MONOCLONAL ANTIBODIES TO STEM CELL FACTOR RECEPTORS

BACKGROUND OF THE INVENTION

5   The present invention relates to monoclonal antibodies specific for a cell receptor that binds human stem cell factor (hSCF), as well as pharmaceutical compositions containing such monoclonal antibodies and uses of such monoclonal antibodies.

10   Stem Cell Factor (SCF) is a growth factor that stimulates the proliferation of pluripotent hematopoietic progenitor cells. It has been produced recombinantly in *E. coli* and various mammalian cells [Zsebo et al., *Cell 63*:195-212 (1990); and co-pending

15   U.S. Patent Applications 07/589,701, 07/573,616, and 07/537,198, filed October 1, 1990, August 24, 1990, and June 11, 1990, respectively].

    The proto-oncogene c-*kit* has recently been identified as the receptor for SCF [Zsebo et al.,

20   *Cell 63*:213-224 (1990)]. Prior to identification of c-*kit* as the ligand for SCF, the c-*kit* receptor was known to exist [Yarden et al., *EMBO J. 6*:3341-3351 (1987); Qiu et al., *EMBO J. 7*:1003-1011 (1988); Flanagan and Leder, *Cell 63*:185-194 (1990)].

25   Polyclonal antibodies directed against the murin c-*kit* have be n reported [C llular Biology

8:4896-4903 (1988)], but it is not known whether these antibodies will cross react with the human c-*kit*, whether they will block binding of SCF to its receptor, or whether they will affect cell growth. A polyclonal antibody raised against a human c-*kit* carboxy terminal peptide has also been reported [*EMBO J.* 6:3341-3351 (1987)], but these antibodies would not block SCF binding to the receptor. A monoclonal antibody that recognizes human SCF receptors has been reported [Lerner et al., *Blood* 76 (Suppl):295a, (1990); Ashman et al., *Leukemia Res.* 12:923-928 (1988); Cambaseri et al., *Leukemia Res.* 12:929-939 (1988); Gadd and Ashman, *Leukemia Res.* 11:1329-1336 (1985)].

Thus, until the existence of the present invention, the prior art has not been able to obtain a monoclonal antibody to the c-*kit* receptor with any expectation that such a monoclonal antibody would possess the ability to block the binding of the c-*kit* ligand, SCF.

SUMMARY OF THE INVENTION

The present invention relates to a monoclonal antibody comprising a monoclonal antibody having an ability to bind to an SCF receptor. Preferably, the binding of the monoclonal antibody to the SCF receptor will also inhibit binding of an SCF molecule to said SCF receptor. Preferably, the SCF and the SCF receptor will be of human origin.

In another aspect of the present invention, the SCF receptor monoclonal antibodies are used in a method of purifying hematopoietic cells comprising the steps of:

(a)   exposing a mixture of cells to such monoclonal antibodies;

(b)   separating cells that bind to said monoclonal antibodies from cells that do not bind to said monoclonal antibodies.

In another aspect of the present invention, the hematopoietic cells purified with the SCF receptor monoclonal antibodies are used in a method of reconstituting hematopoietic cells comprising bone marrow transplantation.

In another aspect of the present invention, the hematopoietic cells purified with the SCF receptor monoclonal antibodies are used in a method of gene

therapy comprising retrovirally-mediated gene transfer into the purified cells.

Another aspect of the present invention relates to a method of separating normal cells from

5   neoplastic cells comprising the steps of:

(a)   exposing a mixture of cells comprising normal cells and neoplastic cells to a monoclonal antibody according to the present invention;

(b)   separating normal cells from neoplastic

10   leukemia cells based upon a differential in numbers of SCF receptors on normal cells and neoplastic leukemia cells.

Another aspect of the present invention relates to use of the SCF receptor monoclonal antibodies

15   for treating neoplastic cells by administration of a therapeutically effective amount of an anti-neoplastic therapeutic agent conjugated to such a monoclonal antibody.

The present invention also relates to a method of

20   treating neoplastic cells comprising administration of a therapeutically effective amount of a neoplastic therapeutic agent conjugated to a binding fragment of a monoclonal antibody of the present invention.

Another aspect of the present invention

25   relates to a method of determining the presence of SCF receptors in a cell sample comprising th steps of:

(a)   exposing a cell sample to a monoclonal antibody of the present invention;

(b)   detecting the binding of said monoclonal antibody to SCF receptors.

5   The monoclonal antibodies of the present invention are also useful as a method of modifying sensitivity to cell cycle-specific chemotherapeutic agents comprising administration of a SCF-inhibiting amount of a monoclonal antibody of the present

10   invention.

DESCRIPTION OF THE DRAWINGS

Figure 1:   Scatchard analysis of ^{125}IhSCF

15   binding to human fetal liver cells.   0.9 X 10^6 fetal liver cells were incubated with ^{125}IhSCF (5 picomolar to 2 nanomolar) and 100 fold excess unlabelled hSCF for 4 hours at 15°C.

Figure 2:   Effect of recombinant human SCF

20   (rHuSCF)on the growth of acute nonlymphocytic leukemia cells when administered alone or in combination with other growth factors such as interleukin-3.

Figure 3:   Scatchard plot of ^{125}IhSCF binding to blasts from a patient with acute nonlymphocytic

25   leukemia (ANLL).

Figure 4: Scatchard analysis of ^{125}IhSCF binding to human small cell lung cancer cells. 0.2 x 10^6 small cell lung cancer cells (H69 cell line) were incubated with ^{125}IhSCF (5 picomolar to 2 nanomolar) and

5   100 fold excess unlabelled hSCF for 4 hours at 15°C.

Figure 5: Scatchard plot of ^{125}IhSCF binding to OCIM1 cells.

Figure 6: Indirect immunofluorescence analysis of SCF binding to normal human bone marrow.

10   The bone marrow cells were simultaneously labelled with anti-CD34 monoclonal antibody and with either SR-1 (Figure 7A) or with an isotype matched control monoclonal antibody (anti-Thy 1.1), (Figure 7B).

Figure 7: Recognition of the SCF receptor,

15   c-*kit*, by monoclonal antibody SR-1. COS-1 cells were transfected with V19.8 or V19.8 containing human c-*kit*. The COS cell membranes were incubated with 1 nanomolar ^{125}IhSCF with or without cold SCF or SR-1 ascities (diluted 1:1000) and bound labelled SCF was measured.

20

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a monoclonal antibody comprising a monoclonal antibody having an

25   ability to bind to an SCF receptor. Preferably, the binding of the monoclonal antibody to the SCF receptor

will also inhibit binding of an SCF molecule to said SCF receptor. Preferably, the SCF and the SCF receptor will be of human origin. More preferably, the monoclonal antibody will be of the IgG2a isotype.

5   Such a monoclonal antibody can be obtained by general methods, including the steps of immunizing or sensitizing an animal with an antigen or immunogen, obtaining the antibody-producing cells resulting therefrom, fusing such antibody producing cells to a

10   stable and long living cell line (an immortal cell line) to produce hybridomas, screening the hybridomas to select a colony consisting of cells that produce the desired antibody, and isolating the resulting monoclonal antibody from such cells.

15   Sensitization can be accomplished by injecting the antigen into an antibody producing species. Preferably the injection will be into a mammal and more preferably into mice. Usually an initial injection is given followed by subsequent booster injections to

20   maximize the response. Optimally, the injection regime is in multiple doses given to Balb/C mice, e.g., one injection intraperitoneally per week for three consecutive weeks. The amount of antigen injected must be adequate to elicit a sufficient amount of antibody to

25   be detectable. Preferred amounts of antigen to be injected are 10^4 to 10^8 cells containing SCF receptors,

preferably 10^5 to 10^7 cells containing SCF receptors,
most preferably about 10^6 cells containing SCF
receptors.

In addition, the generation of human
5 monoclonal antibodies can be performed using *in vitro*
immunization techniques [Ho et al., *J. Immunol. 135*:3831
(1985)]. The variable region of the mouse monoclonal
antibody can also be genetically engineered onto the
constant region of a human immunoglobulin which may be
10 preferable for use in humans to prevent problems of
immunogenicity often associated with administration of
foreign proteins to humans.

These so called "chimeric antibodies" can
be obtained by splicing genes encoding the variable
15 antigen-binding regions of a human antibody molecule
to the constant regions of a human antibody molecule
[Sahagan et al., *J. Immunol. 137*:106601074 (1986);
Beidler et al., *J. Immunol. 141*:4053-4060 (1988);
Morrison et al., *Ann. N.Y. Acad. Sci. 507*:187-198
20 (1988)].

A further refinement envisioned within the
present invention is production of chimeric antibodies
containing a murine hypervariable region coupled to
human constant and framework variable regions [Reichman
25 et al., *Nature 332*:323-327 (1988)]. Most antigen
specificity resides in defined segments of the V regions

(hypervariable regions) or CDR regions (complementary-determining regions). Antigen-combining sites are formed by CDR loops extending from the remaining framework portions of the V regions. Host immune

5   responses may be generated against the less variable rodent framework V regions of chimeric antibodies. Chimeric antibodies containing human framework V regions retain the antigen binding specificity conferred by the murine CDR regions but are unlikely to elicit a host

10 immune response. Total gene synthesis is the most practical method of preparing CDR-replaced variants in which CDRs from a rodent antibody are transplanted into a human framework. Following sequencing of the desired V region, the sequence is chemically synthesized,

15 cloned, and then inserted into an appropriate expression vector.

The antigens that are useful in producing the monoclonal antibodies of the present invention are any cell line that displays an SCF receptor on its surface.

20 Such cell lines include the human erythroleukemia cell lines OCIM1 [Papayannopoulou et al., *Blood* 72:1029-1038(1988)], K562 (ATCC CCL 243); the myeloid or monocytic cell lines KG1 (ATCC CCL 246), KG1α (ATCC CCL 246.1), AML-193 [Santoli et al., *J. Immunology* 139:3348,

25 (1987)], U937 (ATCC CRL 1593); the lymphoid cell lines Daudi (ATCC CCL 213), IM-9 (ATCC CCL159); mast cell line

HMC-1, [Butterfield et al., *Leukemia Research* 12:345
(1988)]; bladder carcinoma cell lines 5637 (ATCC HTB9),
COS (ATCC CRL 1650), BHK (ATCC CCL 10); the gastric
carcinoma cell line KATO3 (ATCC HTB103); the small cell
carcinoma lines H69 (ATCC HTB 119), H128 (ATCC HTB 120);
and the breast carcinoma cell line DU4475 (ATCC HTB
123), which have been deposited with the American Type
Culture Collection, Rockville, Maryland. Preferred
antigens are the human erythroleukemia cell line OCIM1.

As a result of the sensitization process, the
sensitized animal will produce B-cells that produce and
secrete antibodies specific for the antigen. Such cells
can be isolated for further use by removing the spleen
of the immunized mouse

The antibody producing cells thus obtained can
then be fused to a suitable stable and long living cell
line (immortal cell line) using techniques that are
known in the art [Kohler and Milstein, *Nature*
256:495-497 (1975)]. Suitable cell lines for fusion to
the antibody producing cells are any cell line that
lacks the ability to synthesize antibodies, preferably
also lacking in the ability to grow on medium containing
a selection agent, most preferably possessing the mutant
hypoxanthine-guanidine phosphoribosyl transferase gene
(HGPRT- gene), which cannot produce the active
hypoxanthine-guanidine phosphoribosyl transferase

protein. Hypoxanthine-guanidine phosphoribosyl transferase is necessary to grow on a medium containing aminopterin. Such cell lines that are preferred include myeloma cells, more preferably the NS-1 murine myeloma

5 cell line [ATCC T1B 18; Nowinski et al., *Virology* *93*:111-126 (1979)]. Recently, there has even been success in using human cell lines as fusion partners [Banchereau et al., *Science 251*:70-72 (1991)].

The resulting fusion partners can then be

10 screened to select a colony consisting of cells that produce the desired antibody. Screening techniques are known in the art, and usually involve the growing of the fused cells on a medium containing a selection agent that (1) would lead to the death of the unfused immortal

15 cells when such immortal cells lack the ability to circumvent the selection agent but (2) allow growth of cells containing genetic material from the antibody producing cell when such genetic material contains the potential to circumvent the selection agent. A

20 preferred immortal cell line contains the HGPRT- gene and a preferred medium contains aminopterin, more preferably the medium hypoxanthine aminopterin thymidine (HAT). As a result, only the fusion cells having both the HGPRT+ gene from the antibody producing cell line

25 and the characteristic of immortality from the immortal cell line would survive and grow in the medium.

The successful fusion cells, or hybridomas, can then be screened to determine if they have the ability to produce antibodies to the antigen used for sensitization. In the case of SCF, such screening can be by the ability of the hybridoma products to bind to SCF receptors, the ability of the hybridoma products to inhibit binding of SCF to SCF receptors, or by standard immunological techniques (e.g., immunoprecipitation of radiolabelled purified SCF receptor) or by ability of the hybridoma products to recognize purified SCF receptor in an ELISA assay. Preferably, the hybridomas can be screened by the ability of hybridoma products to block binding of SCF to SCF receptors.

Stem cell factors (SCFs) useful in these assays include any of the SCFs from various species. Such SCFs are usually in solution with a suitable adjuvant, which adjuvant may contain buffers, salts, etc. Preferably, the SCF will be a human SCF (HuSCF), more preferably a recombinant human SCF (rHuSCF), and most preferably a rHuSCF produced in *E. coli*. Such SCFs can be obtained as previously described [Zsebo et al., *Cell 63*:195-212 (1990); and co-pending U.S. Patent Applications 07/589,701, 07/573,616, and 07/537,198, filed October 1, 1990, August 24, 1990, and June 11, 1990, respectively, all of which are hereby incorporated by reference for their relevant teachings].

Those hybridomas that are positive for secretion of antibodies to the SCF receptor can then be subcloned and essentially maintained indefinitely. Such selected hybridomas can also be cultured for the

5   production of the monoclonal antibodies that they secrete. The desired monoclonal antibody can be isolated from a culture of such hybridomas using techniques that are known in the art, including protein A-sepharose column chromatography [Ey et al.,

10   *Immunochemistry 15*:429 (1978)].

(3)   The preferred monoclonal antibodies of the present invention are those designated SR-1, deposited as BA7.3C.9 with the American Type Culture Collection, Rockville, Maryland, USA on April   1991, and given

15   the Accession Number _____.

The monoclonal antibodies of the present invention can be used in a method of purifying hematopoietic cells comprising the steps of:

(a)   exposing a mixture of cells to such

20   monoclonal antibodies;

(b)   separating cells that bind to said monoclonal antibodies from cells that do not bind to said monoclonal antibodies.

The exposure of a cell mixture to such

25   monoclonal antibodies can be in solution, as is the case with fluorescence-activated cell sorting, or it can be

with the monoclonal antibody immobilized on a solid
support, such as is the case with column chromatography
or direct immune adherence. In addition, a combination
of soluble and solid support monoclonal antibodies can

5   be used to expose the cell mixture to such monoclonal
antibodies, as has been the case with anti-CD34 antibody
and a biotinylated second antibody put through an avidin
column to remove breast cancer cells in human
transplants [Bensinger et al., *J. Clin. Apheresis*

10   5:74-76 (1990); Berenson et al., *Blood 76*:509-515
(1986)].

        The mixture of cells that is to be exposed to
the monoclonal antibody can be any solution of bone
marrow cells, blood cells or tissue cells. Preferably,

15   the cell mixture is from mammalian bone marrow,
circulating blood, or suspected tumor tissue. After
exposure of the cell mixture to the monoclonal antibody,
those cells with SCF receptors will bind to the
monoclonal antibody to form an antibody-SCF-receptor-

20   cell complex. Such SCF receptor cell complexes can then
be separated from noncomplexed cells by methods that are
known in the art. Preferred methods of separation
include column chromatography, fluorescence-activated
cell sorting, magnetic bead separation, and direct

25   immune adherenc .

The hematopoietic cells thus purified can be employed in a method of reconstituting hematopoietic cells comprising bone marrow transplantation. Methods of bone marrow transplantation are known in the art

5 [Hill et al., *Bone Marrow Transplant.* 4:69-74 (1989)], but heretofore it has not been possible to use such a homogeneous population of cells having SCF receptors as the material transplanted. Such cells are responsible for long term engraftment in a bone marrow transplant

10 and can be separated from contaminating tumor cells that may be present in the bone marrow using the methods described above. Moreover, the cells having SCF receptors purified by the purification method of the present invention can be further subfractionated to

15 obtain even more homogeneous cell populations. For example, a population of SCF-receptor-containing cells can be sequentially exposed to monoclonal antibodies specific for other cell surface proteins that occur on only certain subpopulations of the SCF-receptor-

20 containing cells. Examples of other monoclonal antibodies that can be used in such a sequential method of purification include monoclonal antibodies to the CD34 antigen which is also expressed on hematopoietic stem cells [Andrews et al., *J. Exp. Med.* 169:1721-1731

25 (1989); Civin, United States Patent 4,965,204, issued

October 23, 1990; Civin, European Patent Application
395355, published October 31, 1990].

Hematopoietic cells purified according to the
present invention can also be used in a method of gene
5   therapy comprising retrovirally-mediated gene transfer
into the purified cells. Methods of retrovirally-
mediated gene transfer are known in the art [Bodine et
al., *Proc. Natl. Acad. Sci. USA 86*:8897-8901 (1989)],
but heretofore it has not been possible to use such a
10   homogeneous population of cells having SCF receptors as
the cells transfected. Such transfected cells can then
be used in bone marrow transplantation.

The present invention also relates to a method
of separating normal cells from neoplastic cells
15   comprising the steps of:

(a)   exposing a mixture of cells comprising
normal cells and neoplastic cells to a monoclonal
antibody according to the present invention;

(b)   separating normal cells from neoplastic
20   leukemia cells based upon a differential in numbers of
SCF receptors on normal cells and neoplastic leukemia
cells. The bone marrow cells can be labelled with the
monoclonal antibodies of the present invention, then
with a biotinylated goat anti-mouse antibody and passed
25   through an avidin column. This approach can be used to
positively or negatively select cells; cells with higher

numbers of SCF receptors will be retained by the column while cells with lower SCF receptor display will pass through the column. Alternatively, a method for separating cells with high and low SCF receptor display

5   is direct immune adherence and fluorescence-activated cell sorting. With fluorescence-activated cell sorting (FACS), cells displaying SCF receptors can be mixed with monoclonal antibodies specific for SCF receptors. The monoclonal antibodies are (1) conjugated with a

10   fluorescence agent such as fluoresceine isothiocyanate (FITC) or phycoerythrin (PE); (2) conjugated with a first biological molecule (e.g., biotin) and mixed with a second biological molecule that specifically binds to the first biological molecule (e.g., avidin or

15   streptavidin), the second biological molecule being conjugated with a fluorescent agent such as FITC or PE; or (3) further mixed with a second antibody specific for the species of antibody of the anti-SCF receptor monoclonal antibody (e.g., a goat or sheep anti-mouse

20   antibody), the second antibody being conjugated with a fluorescent agent such as FITC or PE. Such fluorescently labelled cells can then be sorted using standard technology according to the level of fluorescence exhibited by the cells.

25   The monoclonal antibodies of the present invention can also be useful in treating neoplastic

cells by administration of a therapeutically effective

amount of an anti-neoplastic therapeutic agent

conjugated to such a monoclonal antibody. A

therapeutically effective amount of a neoplastic

5   therapeutic agent is any amount of a compound that will

cause inhibition of growth and/or development of

neoplastic cells, preferably causing death of the cell

and a decrease in the total number of neoplastic cells

in an organism. Examples of such neoplastic therapeutic

10   agents include antibodies coupled to the radioisotope

^{125}I [Press et al., *J. Clin. Oncol.* 7:1027-1038 (1989)]

or to toxin conjugates such as ricin [Uhr et al., *Prog.*

Clin. Biol. Res. 288:403-412 (1989)] and the diptheria

toxin [Moolten, *J. Natl. Con. Inst.* 55:473-477 (1975)].

15   Conjugation of the leukemia therapeutic agent

to the monoclonal antibody can be accomplished using

known techniques as described above [Press et al.,

J. Clin. Oncol. 7:1027-1038 (1989); Uhr et al., *Prog.*

Clin. Biol. Res. 288:403-412 (1989)]. Preferably, the

20   conjugation site on the monoclonal antibody is at a

location distinct from the binding site for the

monoclonal antibody to the SCF receptor. It is also

preferred that the conjugation site on the neoplastic

therapeutic agent be at a functional group distinct from

25   the active site of the therapeutic agent. More

preferably, the conjugation site will also be situated

so as to minimize conformational changes of the monoclonal antibody or the neoplastic therapeutic agent.

The present invention also relates to a method of treating neoplastic cells comprising administration of a therapeutically effective amount of a neoplastic therapeutic agent conjugated to a binding fragment of a monoclonal antibody of the present invention. Suitable binding fragments are those fragments that retain sufficient size and structure to allow binding of the fragment to the SCF receptor. Such fragments can be prepared by numerous methods, including proteolytic digestion [Garvey et al., *Methods in Immunology*, Chapter 31, W.A. Benjamin, Reading, Massachusetts (1977)]. The prepared binding fragments can be assayed for ability to bind to the SCF receptor using the binding assays previously described.

Another use of the monoclonal antibodies of the present invention relates to a method of determining the presence of SCF receptors in a cell sample comprising the steps of:

(a) exposing a cell sample to a monoclonal antibody of the present invention;

(b) detecting the binding of said monoclonal antibody to SCF receptors.

The exposure of a cell mixture to such monoclonal antibodies can be in solution, as is the case

for fluorescence-activated cell sorting, or it can be on solid tissue specimens such as biopsy material, or it can be with the monoclonal antibody immobilized on a solid support, as is the case with column chromatography

5 or direct immune adherence. The mixture of cells that is to be exposed to the monoclonal antibody can be any solution of blood cells or tissue cells. Preferably, the cell mixture is from normal mammalian cells, mammalian bone marrow, circulating blood, or suspected

10 tumor tissue, more preferably normal cells, leukemia cells and solid tumor cells. After exposure of the cell mixture to the monoclonal antibody, those cells with SCF receptors will bind to the monoclonal antibody to form an antibody-SCF-receptor complex. The presence of the

15 antibody-SCF-receptor complex, and therefore SCF receptors, can be detected by methods known in the art. These methods include ELISA, immunohistochemistry, and autoradiography using ^{125}C-labelled Staph Protein A.

The monoclonal antibodies of the present

20 invention are also useful as a method of modifying sensitivity to cell cycle-specific chemotherapeutic agents comprising administration of a SCF-inhibiting amount of a monoclonal antibody of the present invention. An SCF-inhibiting amount of a monoclonal

25 antibody is sufficient quantities of monoclonal antibody to significantly inhibit the binding of SCF to its

receptor or to significantly decrease the growth and
development of cells containing the SCF receptor, e.g.,
early pluripotent hematopoietic progenitors, leukemia
cells, solid tumor cells, bone marrow cells. Generally,

5   a significant inhibition is inhibition that is larger
than the variance due to error expected with a given
method of measuring the inhibition. Preferably, the
inhibition will decrease binding of SCF to its receptor
by at least 50%, more preferably by at least 75%, more

10 preferably by at least 90%, and most preferably
inhibition will decrease binding of SCF to its receptor
essentially entirely. Generally, a significant decrease
of the growth and/or development of cells containing the
SCF receptor is a decrease larger than the variance due

15 to error expected with a given method of measuring the
growth and/or development. Preferably, decrease of the
growth and/or development of cells containing the SCF
receptor is a lowering of the growth rate of SCF-
receptor-containing cells, preferably a decrease to at

20 least one-half, more preferably to at least one-tenth,
and most preferably to at least one-hundredth.

Administration of the monoclonal antibodies of
the present invention involves administration of an
appropriate amount of a pharmaceutical composition

25 containing the monoclonal antibodies as an active
ingredient. In addition to the active ingredient, the

pharmaceutical composition may also include appropriate

buffers, diluents and additives. Appropriate buffers

include Tris-HCl, acetate, glycine and phosphate,

preferably phosphate at pH 6.5 to 7.5. Appropriate

5   diluents include sterile aqueous solutions adjusted to

isotonicity with NaCl, lactose or mannitol, preferably

NaCl. Appropriate additives include albumin or helatin

to prevent adsorption to surfaces, detergents (e.g.,

Tween 20, Tween 80, Pluronic F68), solubilizing agents

10   (e.g., glycerol, plyethylene glycol), antioxidants

(e.g., ascorbic acid, sodium metabisulfite) and

preservatives (e.g., Thimersol, benzyl alcohol,

parabens). A preferred additive is Tween 80.

Administration may be by any conventional

15   means including intravenously, subcutaneously, or

intramuscularly. The preferred route of administration

is intravenous. Administration may be a single dose or

may occur in an appropriate number of divided doses.

Preferably, the pharmaceutical preparation is

20   in unit dosage form. In such form, the preparation is

subdivided into unit doses containing the appropriate

quantities of the active component, e.g., an effective

amount to achieve the desired purpose.

The actual dosage employed may be varied

25   depending upon the requirements of the patient and the

severity of the condition being treated. Determination

of the proper dosage for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is

5 increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered essentially continuously or in portions during the day if desired. The amount and frequency of administration

10 will be regulated according to the judgment of the attending clinician considering such factors as age, condition and size of the patient as well as severity of the disease being treated.

A typical recommended dosage regime for use in

15 the present invention is from about 0.1 to about 10 mg active ingredient per kg body weight per day.

EXAMPLES

20 The following examples are intended to illustrate specific embodiments of the present invention without limiting the scope thereof. All references cited are hereby incorporated by reference for their relevant teachings.

25

Example 1: Sensitization of Animals.

Appropriate antigens for use in sensitization were any cell displaying SCF receptors. The presence of

5   SCF receptors was determined using radiolabelled SCF. Human and rodent SCF[164-165] was obtained according to the methods of Zsebo et al., *Cell 63*:195-212 (1990); and copending U.S. Patent Applications 07/589,701, 07/573,616, and 07/537,198, filed October 1, 1990,

10 August 24, 1990, and June 11, 1990, respectively. These SCFs were labelled with ^{125}I using the chloramine-T method of Hunter and Greenwood [*Nature 194*:495-496 (1962)]. The specific activity of the ^{125}I human SCF (hSCF) varied from 2,000 to 2,500 Ci/mmol. Both ^{125}I

15 hSCF and ^{125}I rat SCF (rSCF) retained the ability to bind to SCF-receptor-containing cells. Moreover, self displacement analysis [Calvo et al., *Biochem. J. 212*:259-264 (1983)] with ^{125}IhSCF and unlabelled hSCF demonstrated that the binding affinity was not altered

20 by iodination. A number of other hematopoietic growth factors were tested for binding to the erythroleukemia cell line OCIM1 [Papayannopoulou et al., *Blood 72*:1029-1038 (1988)]. Table 1 shows that a 100-fold molar excess of unlabelled hSCF competed very

25 effectively for binding, while a variety of other growth factors did not.

TABLE 1

COMPETITION WITH ^{125}IhSCF FOR BINDING TO OCIM1 CELLS[a]

Competitor	CPM Bound
NONE	1725
SCF	10
IL-3	1830
GM-CSF	1742
ERYTHROPOIETIN	1775
G-CSF	1843
IL-6	1693

[a]OCIM1 cells were incubated with 200 picomolar ^{125}I hSCF with or without a 100 fold excess of the growth factors indicated.

Numerous normal hematopoietic cells, hematopoietic cell lines and neoplastic nonhematopoietic cells were screened for expression of SCF receptors. Normal human marrow mononuclear cells bind hSCF, as do human fetal liver early erythroblasts. Adult late erythroblasts, which were obtained by culturing peripheral blood BFU-E and plucking individual colonies after 14 days, also displayed SCF receptors. Distribution of SCF receptors on normal human marrow cells were determined by autoradiography [Nicola and Metcalf, *J. Cell Physiol.* *124*:313-321 (1985)]. Large

cells with high nuclear/cytoplasmic ratio that appeared to be blasts and promyelocytes were densely labelled with approximately 50 to 200 grains per cell. Megakaryocytes also showed ^{125}I binding.

5   A number of human hematopoietic cell lines displayed SCF receptors. The erythroleukemia cell lines OCIM1 and K562 bind SCF, as do the myeloid of monocytic cell lines KG-1, KG1a, AML-193 and U937. The lymphoid cell lines Daudi and IM-9 and the mast cell line HMC-1

10   all bound SCF. SCF receptors were also found on nonhematopoietic cell lines including the bladder carcinoma line 5367, COS, BHK, the gastric carcinoma cell line KATO3, the small cell carcinoma cell lines H69 and H128, and the breast carcinoma cell line DU475.

15   SCF receptors were quantitated on normal human fetal liver cells and examined for their response to SCF in colony assays. Human fetal liver cells (gestational age 55 to 80 days) were obtained from therapeutic abortions. Consent was obtained for the use of these

20   tissues, and the studies were approved by the Institutional Review Board at the University of Washington. The cells were incubated with ^{125}IhSCF (5 picomolar to 2 nanomolar) $\pm$ a 100 fold excess of unlabelled SCF for 4 hours at 15°C in the presence of

25   metabolic inhibitors. Under these conditions, the equilibrium binding for SCF is achieved and

internalization is minimal (<17%). At the conclusion of
the incubation period, cell-associated ^{125}IhSCF was
separated from free ^{125}IhSCF by sedimenting the cells
through phthalate oil, as described in Broudy et al.,

5   Blood 75:1622-1626 (1990). Equations for 1 or 2 classes
of receptors were fitted to the data using a ligand
program [Munson and Rodbard, *Analyt. Biochem.*
107:220-239 (1980)]. The human fetal liver cells were
found to express 2 classes of SCF receptors as shown in

10   Figure 1. The high affinity receptor had a Kd of
14 picomolar and the low affinity receptor had a Kd of
2.7 nanomolar with approximately 1,700 receptors/cell.

       Neoplastic hematopoietic cells were
investigated to determine whether they would also

15   respond to SCF and display SCF receptors. Marrow
mononuclear cells from 20 different patients with acute
nonlymphocytic leukemia (ANLL) at first presentation and
two normal adults were studied. The cells were cultured
in agar supplemented with 15% fetal calf serum and

20   recombinant human IL-3. Colonies (>40 cells) and
clusters (<40 cells) were counted after 8, 15, and 21
days. SCF receptors were quantified by equilibrium
binding studies with ^{125}IhSCF ± a 100 fold excess of
unlabelled hSCF. The cellular distribution of SCF

25   receptors was examined by autoradiography. SCF
stimulated colony growth from 7 of the 20 ANLL marrows

studied and from both of the normal marrows. SCF alone
had little effect on colony growth, but acted
synergistically with IL-3 to increase both the number
and size of colonies (Figure 2). Receptors for SCF were

5   identified on the blasts of all 20 ANLL patients. Ten
of the 20 ANLL patients exhibited 2 classes of SCF
receptors on their marrow blasts. A Scatchard plot of
[125]IhSCF binding to the blasts from one of the ANLL
patients shows approximately 500 high affinity SCF

10 receptors (Kd 16 picomolar) and 7000 low affinity
receptors (Kd 7.6 nanomolar) per cell as illustrated in
Figure 3. These binding affinities are similar to those
found on normal human fetal liver cells and normal human
marrow mononuclear cells. Six of the 20 patients showed

15 a single class of high affinity receptors, while the
remaining patients showed a single low affinity binding
site. Neither the number of receptors/cell nor the
presence of 1 or 2 classes of receptors correlated with
growth response to SCF, as has been observed for IL-3,

20 GM-CSF and G-CSF receptors on human ANLL blasts [Park
et al., *Blood* 74:56-65 (1989)].

The marrow mononuclear cells from these
leukemic patients were greater than 90% blasts while
marrow mononuclear cells from normal adults contain a

25 much lower fraction of blasts. The vast difference in
the percentage of blasts suggests that it is not

accurate to compare the average number of receptors per cell on normal and leukemic marrow samples. Autoradiography, which permits analysis of binding to individual cells, can more accurately be used to compare

5   SCF binding to normal and leukemic blasts. Autoradiographic analysis of [125]IhSCF binding to normal human marrow mononuclear cells on 8 of the ANLL marrow samples was carried out in a single experiment to permit direct comparison. Grain counts indicated that the

10   normal marrow blasts displayed approximately 50 to 200 grains/blast, while the leukemic blasts exhibited from 2 to 20 grains/blast. Thus binding of SCF to leukemic blasts was substantially lower than binding of SCF to normal blasts.

15   SCF receptors were also found on tumor cell lines of non-hematopoietic origin including H69, H128, and DU475. A Scatchard plot of [125]IhSCF binding to H69 cells (Figure 4) shows 1650 high affinity receptors per cell (Kd 37 picomolar) and 22,300 receptors per cell (Kd

20   7.2 nanomolar).

       OCIM1 is a human erythroleukemia cell line that displays receptors for erythropoietin [Broudy et al., *Proc. Natl. Acad. Sci. USA* 85:6513-6517 (1988)], GM-CSF, and IL-3. Equilibrium binding studies with

25   [125]IhSCF showed that OCIM1 cells display about 200,000 SCF receptors per cell as shown in Figure 5. A single

class of high affinity SCF receptors (Kd 45 picomolar)
is evident. hSCF did not stimulate the growth of OCIM1
cells in suspension culture. MB-02 is a growth factor
dependent human erythroleukemia cell line that will

5 undergo erythroid differentiation in the presence of
erythropoietin [Perrine et al., *Biochem. Biophys. Res.
Comm.* *164*:857-862 (1989)]. MB-02 cells respond to hSCF
with proliferation, but not erythroid differentiation,
and display both high and low affinity SCF receptors.

10 The OCIM1 cells were used as an immunogen
because of their high SCF receptor display, although any
cell displaying SCF receptors could be used as an
immunogen to elicit antibodies to the SCF receptor.

Eight week old female Balb/C mice were
15 injected intraperitoneally with 10^6 OCIM1 cells on three
occasions at one week intervals.

Example 2: Production of
Monoclonal Antibody to SCF Receptor.

20

Five days following the third injection, the
spleen was removed and splenic cells were fused with
NS-1 murine myeloma cells [Nowinski et al., *Virology*
93:111-126 (1979)]. The supernatants from a total of
25 288 hybridoma wells were screened for the ability to
block binding of ^{125}IhSCF to OCIM1 cells as described in

Example 5, below. A positive hybridoma was identified,
cloned and grown as an ascites-producing tumor in
pristane-primed Balb/C mice. The antibody was
identified as IgG2a and was named SR-1 (deposited as

5   BA7.3C.9 with the American Type Culture Collection,
Rockville, Maryland USA on April   , 1991 and given the
ATCC Accession Number _____ . Screening of additional
hybridomas should lead to the identification of
additional anti-SCF receptor monoclonal antibodies at a

10   similar frequency.

Example 3: Production of Chimeric Monoclonal Antibodies Having Murine Variable Regions and Human Constant Regions.

15

Genomic DNA is prepared from the hybridoma
cell line producing SR-1 monoclonal antibodies, and
functional exons encoding the variable regions of heavy
and light chains (V_H and V_κ, respectively) are

20   identified by DNA restriction maps obtained by Southern
analysis. Functional V_κ exons result when germline V_κ
genes are rearranged and joined to the J_κ gene segment.
Similarly, a functional V_H exon is created when a V_H
gene is juxtaposed to the J_H gene segment. Specific DNA

25   probe segments are designed to identify rearranged
V-regions genes via unique restriction enzyme sites that

distinguish the rearranged genotype from the
unrearranged germline DNA sequences [Oi and Morrison,
BioTechniques *4*:214-221 (1986)]. Recombinant DNA
techniques are used to construct a genomic DNA library,
5   and the desired V-genes are isolated and sequenced to
confirm the identification of rearranged expressed V_H
and V_L exons. The V_H exon is inserted into the
pSV2ΔHgpt vector [Mulligan and Berg, *Science*
209:1422-1427 (1980); Mulligan and Berg, *Proc. Natl.*
10 *Acad. Sci. USA* *78*:2072-2076 (1981)], which contains an
ampicillin resistance gene to maintain the plasmid in
E. coli, and a mycophenolic acid resistance gene to
permit selection in mammalian cells growing in medium
containing hypoxanthine, mycophenolic acid and xanthine.
15 The desired V_K exon is inserted into the pSV184ΔHneo
vector, which is derived from the pACYC184 plasmid
[Chang and Cohen, *J. Bacteriol.* *143*:1141-1156 (1978)].
This vector contains a chloramphenicol resistance gene
used to maintain the plasmid in *E. coli* and a gentamycin
20 resistance gene used to select for mammalian cells
transfected with this plasmid vector. Transcription of
the gentamycin resistance gene (*neo*) is directed by the
SV40 early region promoter. Known DNA regulatory
sequences for the immunoglobulin heavy and light chains
25 are also included in these transfection vectors [Calame,
Annual Rev. Immunol. *3*:159-196 (1985); Morrison and Oi,

Annual Rev. Immunol. 2:239-256 (1984)]. Both plasmids
are maintained in *E. coli* HB101 [Boyer and Roulland-
Dussoix, *J. Mol. Biol.* 41:459 (1969)] grown in
chloramphenicol and ampicillin selection medium.

5   Protoplasts of these bacterial cells, prepared
by treatment with lyzozyme and EDTA, are fused via
polyethylene glycol treatment or electroporation) with
the immunoglobulin nonproducing mouse SP2/0 myeloma cell
line (ATCC CRL 1581). Transfected SP2/0 cells are
10   isolated using medium containing gentamycin,
hypoxanthine, mycophelolic acid and xanthine. The
resulting transfectomas are screened for production of
mouse:human chimeric SR-1 antibodies using the
techniques described in Example 2.

15

Example 4: Assay to Determine Binding of Monoclonal
Antibody SR-1 to SCF Receptor.

COS-1 cells were transfected with a vector
20   containing the transmembrane and external domain of
human c-*kit* [Zsebo et al., *Cell* 63:213-224 (1990)], or
with the vector alone. Indirect immunofluorescence
analysis using SR-1 followed by FITC-conjugated goat
anti-mouse IgG showed that SR-1 recognized none of the
25   cells transfected with vector alone and 5 to 10% of the

cells transfected with c-*kit*. This demonstrates that SR-1 binds to c-*kit*.

Analysis by indirect immunofluorescence also showed that the monoclonal antibody (SR-1) recognizes a

5   surface epitope present on both OCIM1 cells and a small percentage (2-5%) of marrow mononuclear cells (Figure 6A). The SR-1 antibody recognizes a portion (50-75%) of the hematopoietic cells that display CD34 (Figure 6B). These results were obtained when bone marrow cells were

10 simultaneously labelled with anti-CD34 monoclonal antibody (antibody 12.8, Andrews et al., *J. Exp. Med.* 172:355, (1990); Civin, United States Patent 4,965,204, issued October 23, 1990; Civin, European Patent Application 395355, published October 31, 1990) and with

15 either SR-1 or with an isotype matched control monoclonal antibody anti-Thy 1.1, Andrews et al., *J. Exp. Med.* 172:355, (1990). These examples demonstrate that SR-1 and indirect immunofluorescence analysis can be used to identify cells that express

20 c-kit. SR-1 antibodies have also been directly conjugated to PE, and this preparation has been used to identify cells that display c-kit. Alternative methods are biotinylation of the SR-1 antibody, with binding of this preparation detected using avidin or streptavidin

25 conjugated to FITC or PE.

Example 5: Assay to Determine Inhibition of SCF Binding to SCF Receptor by Monoclonal Antibody SR-1.

Cells that express the SCF receptor were
5   incubated with ^{125}IhSCF (100 picomolar) with or without varying quantities of SR-1 antibody. Preferably, a dilution of 1:1000 to 1:100,000 of SR-1 ascites is used. At the conclusion of the incubation, cell associated ^{125}IhSCF was separated from free ^{125}IhSCF by sedimenting
10   the cells through phthalate oil [Broudy et al., *Blood* 75:1622-1626 (1990)]

The ascites blocks binding of ^{125}IhSCF to OCIM1 cells at a 1:100,000 dilution (Table 2). This monoclonal antibody is specific for the human SCF
15   receptor in that it does not block binding of ^{125}I-ratSCF to the murine MC/9 cell line.

20

25

TABLE 2

SR-1 BLOCKS BINDING OF ^{125}I hSCF TO OCIM1 CELLS[a]

Addition	CPM Bound
0	1548
Unlabelled SCF	89
Ascites 1:1,000	70
Ascites 1:10,000	39
Ascites 1:100,000	118
Ascites 1:1,000,000	760
Ascites 1:10,000,000	1375

[a]OCIM1 cells were incubated with 100 picomolar ^{125}IhSCF with dilutions of SR-1 ascites fluid.

COS-1 cells were also transfected with V19.8, Zsebo et al., *Cell* 63:213-224 (1990 or V19.8 containing human c-kit. The COS cell membranes were incubated with 1 nanomolar ^{125}IhSCF with or without cold SCF or SR-1 ascities (diluted 1:1000) and bound labelled SCF measured. SR-1 blocked binding of ^{125}IhSCF to c-kit as effectively as unlabelled SCF (Figure 7).

Example 6: SR-1 Neutralizes the Biologic Effect of SCF.

In addition to blocking the binding of ^{125}IhSCF to cells, SR-1 blocks the biologic effects of SCF on colony growth. SCF stimulates the growth of

early erythroid colony forming cells (BFU-E), and SR-1 blocks this effect. SCF does not alter the growth of more mature erythroid colony forming cells (CFU-E) and SR-1 has no effect on CFU-E growth.

5   Human marrow mononuclear cells were cultured in recombinant human erythropoietin (1 unit/ml, Amgen Inc., Thousand Oaks, California) plus hSCF (50 ng/ml) with or without SR-1 ascites (1:1000 dilution) in semisolid medium. CFU-E were counted on day 7, BFU-E

10   were counted on day 14. Three experiments with duplicate plates were conducted and the results from one such experiment are presented in Table 3.

15   TABLE 3

         EFFECT OF SR-1 ON HUMAN ERYTHROID COLONY GROWTH

SR-1 ANTIBODY	Colonies/10^5 Cells	
	CFU-E	BFU-E
−	39	142
+	38	20

25   Example 7: Conjugation of Monoclonal
         Antibody SR-1 to a Therapeutic Agent.

         SR-1 is coupled or conjugated to a variety of agents, for therapeutic and diagnostic use of the

resulting conjugates, Scheinberg et al., *Oncology 1*, 31-37 (1987).

For use in *in vivo* imaging of tumors and tumor masses containing cells expressing c-*kit* receptor,

5 antibody or antibody fragments are coupled to radioisotopes such as ^{123}I, ^{131}I, ^{111}In, ^{90}Y, ^{99}Tc. For use in therapy of such tumors and in therapy of dispersed malignancies such as leukemias, antibody or antibody fragments are coupled to radioisotopes such as

10 ^{32}P, ^{131}I, ^{90}Y, ^{186}Re, ^{212}Pb, ^{212}Bi [Scheinberg et al., *Oncology 1*, 31-37 (1987) and Humm, J. L., *J. Nuclear Medicine* 27, 1490-1497 (1986)]. Conjugation of radioisotopes to antibody is accomplished by direct attachment of radioisotopes to antibody by methods that

15 include pertinning techniques [Schwartz J., *Nuclear Medicine* 28, 721 (1987) and Rhodes et al., *J. Nuclear Medicine* 21, 54 (1980)]; or by way of bifunctional chelate linkers such as those utilizing diethylenetriaminepentaacetic acid (DTPA) [Hnatowich

20 et al., *J. Nuclear Medicine* 26:503-509 (1985)], N_2S_2 [Fritzberg et al. *Proc. Natl. Acad. Sciences U.S.A.* 85:4025 (1988)], or macrocyclic chelators [Moi et al., *Cancer Research* (Suppl.) 50:7895-7935 (1990)], which bind both antibody and radioisotope.

25 For use in therapy, a variety of other toxic agents are attached to antibody. These include

antitumor drugs and antibiotics which are toxic by way

of interaction with DNA via intercalation (e.g.,

daunomycin, adriamycin, aclacinomycin) or cleavage of

DNA (e.g., esperamycin, calicheamycin,

5 neocarzinostatin), and other toxic cytostatic drugs such

as cis-platinum, vinblastine, and methotrexate

[Scheinberg et al., *Oncology* 1:31-37 (1987); Greenfield

et al., *Antibody, Immunoconjugates, and*

Radiopharmaceuticals 4:107-119 (1991); Dillman et al.,

10 *Cancer Research* 48:6097-6101 (1988);Hamann et al.,

Abstracts of 197th American Chemical Society National

Meeting, Dallas, Texas, U.S.A., April 9-14, 1989,

Abstract No. 71A; Y. Sugiura et al., *Proc. Natl. Acad.*

Sci. U.S.A. 86:7672-7676 (1989)]. These agents are

15 coupled in ways that include covalent attachment upon

reaction with appropriate derivatives of the agents.

Also for use in therapy, many protein and

glycoprotein toxins are conjugated to antibody [Blättler

et al., *Cancer Cells* 1:50-55 (1989); *Immunotoxins,*

20 Edited by A.E. Frankel Kluwer Academic Publishers,

Boston (1988)]. These include bacterial toxins such as

Diphtheria toxin, Shigella toxin, and Pseudomonas

exotoxin; plant toxins such as ricin, abrin, modeccin,

viscumin, pokeweed antiviral protein, saporin, momordin,

25 and gelonin. The toxins contain a catalytic fragment

and in some cases fragments or domains that recognize

cell surface structures or facilitate translocation across cell membranes. Thus appropriately modified toxins or toxin fragments are used, which permit improved specificity without loss of potency (e.g.,

5 modified toxins which themselves lack the capability for cell surface recognition, so that such recognition is provided only by the antibody to which conjugation is done, but which retain the membrane translocation capability which enhances potency) [Hnatowich et al.,

10 *J. Nuclear Medicine* 26:503-509 (1985)]. Conjugation of toxins to antibody is done by heterobiofunctional cross-linkers such as N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) or 2-iminothiolane [Cawley et al., *Cell* 22:563-570 (1980); Goff et al., *Bioconjugate*

15 *Chemistry* 1:381-386 (1990); and Thorpe et al., *J. Natl. Cancer Inst.*, 79:1011]. In addition, toxins fused to a c-*kit* binding component of antibody are generated by recombinant expression of genetically-engineered elements of toxin and antibody genes joined as a

20 continuous genetic element.

Prior to diagnostic or therapeutic use, conjugated antibodies are tested to judge their toxic potency, target specificity, *in vitro* and *in vivo* stability, and other properties, [Blättler et al.,

25 *Cancer Cells* 1:50-55 (1989) and *Immunotoxins*, Edited by A. E. Frankel Kluwer Academic Publishers, Boston

(1988)]. It is desired that the toxicity of the toxic agent, and the binding affinity and specificity of the antibody, be minimally affected by the coupling procedures used. Thus conjugates are tested for binding

5 to SCF receptor (see Example 3), and inhibition of SCF binding to SCF receptor (see Example 4). *In vitro* toxicity toward target cells such as the erythroleukemia cell line OCIM1 is tested by measuring incorporation of labeled compounds into macromolecules in treated versus

10 untreated cell cultures, and more directly by determining the number of cells in treated versus untreated cultures that are able to grow in clonogenic and cell growth back-extrapolation assays. *In vivo* stability, clearance, and specific toxicity are judged

15 by administration of conjugate to appropriate animal recipients. Such recipients include normal mice and *in vivo* tumor and leukemia xenograft models comprising human neoplastic cells introduced into immunodeficient strains of mice, such as the nude mouse or SCID mouse.

20

25

Example 8: Preparation of Pharmaceutical Composition Containing Monoclonal Antibody SR-1.

5   Pharmaceutical compositions of the present invention include an effective amount of the active ingredient, SR-1, alone or in combination with a suitable buffer, diluent and/or additive. Such compositions are provided as sterile aqueous solutions or as lyophilized or otherwise dried formulations.

10   Typically, antibodies are formulated in such vehicles at concentrations from about 1 mg/ml to 10 mg/ml.

One example of a suitable pharmaceutical composition for injection contains monoclonal antibody SR-1 (1 mg/ml) in a buffered solution (pH 7.0 $\pm$ 0.5) of

15   monobasic sodium phosphate (0.45 mg/ml) and Tween 80 (0.2mg/ml) in sterile H_2O.

Example 9: Selection of Cells Containing SCF Receptors.

20   a. Selection of Cells Containing SCF Receptors by Direct Immune Adherence with SR-1.

Cells expressing SCF receptors were selected by direct immune adherence, and the proliferative potential of these cells was determined in colony

25   assays. Monocyte-depleted normal human marrow mononuclear cells were separated by direct immune

adherence with SR-1, and cultured in erythropoietin plus
IL-3 in semisolid medium for 14 days. The data
represent the average of duplicate plates from 1 of 3
experiments.

5   The results (Table 4) show a 50-fold
enrichment in BFU-E in the SR-1 adherent population of
cells. The fraction of BFU-E ranged from 4-7%, and the
overall recovery of BFU-E in the SR-1 adherent
population was 70%. These results indicate that SR-1
10 can be used to purify populations of progenitor cells by
direct immune adherence.

TABLE 4

15

ISOLATION OF PROGENITOR CELLS WITH SR-1

Cells	Colonies/10^5 Cells	
	BFU-E	CFU-GM
Marrow mononuclear	117	151
SR-1 Adherent	5040	840
SR-1 Non-adherent	6	24

25

          To determine whether more primitive
hematopoietic cells are found in the SR-1 adherent
population, the cells were cultured in suspension for 12
days. At 3 day intervals, aliquots of cells were
30 removed and replated in methylcellulose colony assays to

quantitate progenitors. The results show that the SR-1
adherent cells generated large numbers of BFU-E and
CFU-GM (up to 3-fold above input) throughout the 12 day
suspension culture period. The number of BFU-E and

5   CFU-GM in the SR-1 non-adherent population did not
increase above input, and continuously declined. This
indicates that the SR-1 adherent population of cells
contains more primitive hematopoietic cells that are
capable of generating progenitor cells.

10

      b. Selection of Cells Containing SCF Receptors
by Fluorescence-Activated Cells Sorting with SR-1.

      Normal human bone marrow mononuclear cells
were simultaneously labeled with anti-CD34 (monoclonal

15   antibody 12.8 [Andrews et al., *J. Exp. Med.* 172-355
(1990)] and SR-1, and separated on a FACS as illustrated
in Example 10. The fraction of progenitors was
determined by colony assays in methylcellulose. The
results show that the CD34 positive, SR-1 positive

20   population of cells contained 80% of the BFU-E and 96%
of the CFU-GM. The CD34 positive, SR-1 negative
population of cells contained 20% of the BFU-E and 4% of
the CFU-GM. This indicates that SR-1 identifies a
subset of CD34 positive cells that contains the majority

25   of myeloid and erythroid progenitor cells. Furthermore,

these progenitor cells can be positively selected by
cell sorting with SR-1.

Example 10: Fluorescence Activated Cell

5   Sorting of Cells Displaying SCF Receptors.

In fluorescence activated cell sorting (FACS),
cells displaying SCF receptors were mixed with the
monoclonal antibody SR-1. The SR-1 monoclonal antibody
10   was either (1) conjugated with the fluorescence label
FITC or PE; (2) conjugated with biotin and mixed with
avidin or streptoavidin which is conjugated with FITC or
PE; or (3) further mixed with a goat anti-mouse FITC or
a sheep anti-mouse FITC.
15   Such directly labelled or indirectly labelled
SCF-receptor-containing cells were then separated on
the basis of the level of fluorescence using standard
FACS methods.